Cells, Biomarkers and Post-traumatic Stress Disorder: Evidence for Peripheral Involvement in A Central Disease

James A .Andrews
Kameran D. Neises

Naval Health Research Center

Report No. 10-27

Naval Health Research Center
140 Sylvester Road
San Diego, California 92106

Journal of Neurochemistry

JOURNAL OF NEUROCHEMISTRY | 2012 | 120 | 26–36 doi: 10.1111/j.1471-4159.2011.07545.x

REVIEW

Cells, biomarkers, and post-traumatic stress disorder: evidence for peripheral involvement in a central disease

James A. Andrews and Kameran D. Neises

Naval Health Research Center, San Diego, California, USA

Abstract

Post-traumatic stress disorder (PTSD) is a complicated CNS syndrome. Looking beyond the CNS, recent studies suggest that peripheral blood mononuclear cells could cause and/or exacerbate PTSD. This review summarizes the literature, describes associations between circulating peripheral blood cells and PTSD, proposes a novel mechanism, and analyzes several biomarkers that appear to associate with PTSD symptoms. Several experimental animal models have shown that peripheral blood mononuclear cell activity can cause hippocampal volume loss and PTSD-like symptoms. Data from these models suggest that a traumatic event and/or traumatic events can trigger peripheral cells to migrate, mediate inflammation, and decrease neurogenesis, potentially leading to CNS volume loss. Biomarkers that associate with PTSD symptoms have the potential to differentiate PTSD from traumatic brain injury, but more work needs to be done. Research examining the mechanism of how traumatic events are linked to peripheral blood mononuclear cell functions and biomarkers may offer improved diagnoses and treatments for PTSD patients.

Keywords: biomarkers, PBMC, post-traumatic stress disorder, PTSD.

J. Neurochem. (2012) **120**, 26–36.

Post-traumatic stress disorder (PTSD) is a syndrome characterized as a spectrum of symptoms following exposure to an extremely traumatic stressor. Based on the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision,* diagnostic criteria include re-experiencing the traumatic event, avoidance of stimuli associated with the event, and increased arousal that was not present before the trauma (American Psychology Association 2000). Despite what is known about the symptoms of PTSD, little progress has been made toward describing mechanisms or effective diagnostic biomarkers. Through the study of the CNS, the periphery and common disease mechanisms we can understand the underlying etiology. This review centers on how peripheral blood mononuclear cells (PBMCs) and biomarkers play a role in a putative mechanism and effective diagnosis of PTSD.

PBMCs affecting the CNS

The proposed mechanism is initiated from a traumatic event and terminates with hippocampal volume loss. Figure 1 demonstrates the subsequent stages of microglial activation, inflammation, and decreased neurogenesis. Microglial activation, for the purpose of this paper, refers to cells that have assumed a pro-inflammatory phenotype. Both microglial activation and inflammation are associated with CNS cell death. This increased CNS cell death combined with decreased neurogenesis lead to hippocampal volume loss.

PBMCs, although very important for proper immune function, can also injure healthy neurological tissue, if inflammation progresses unchecked. PBMCs including monocytes, macrophages, and lymphocytes, which are derived from the common hematopoietic stem cell lineage in bone marrow and can be found within the circulatory system. Some studies suggest that the number of circulating

Received May 25, 2011, revised manuscript received October 13, 2011; accepted October 16, 2011.

Address correspondence and reprint requests to James A. Andrews, Department 166, Naval Health Research Center 140 Sylvester Road, San Diego, CA 92106-3521 USA. E-mail:james.andrews@med.navy.mil.

Abbreviations used: BBB, blood–brain barrier; ICAM, intercellular adhesion molecule; IL, interleukin; iNOS, inducible nitric oxide synthase; MRP8, myeloid-related protein-8; NGF, nerve growth factor; NPY, neuropeptide-Y; PBMC, peripheral blood mononuclear cells; PTSD, post-traumatic stress disorder; SDF-1, stromal cell-derived factor-1; TBI, traumatic brain injury; TNF, tumor necrosis factor.

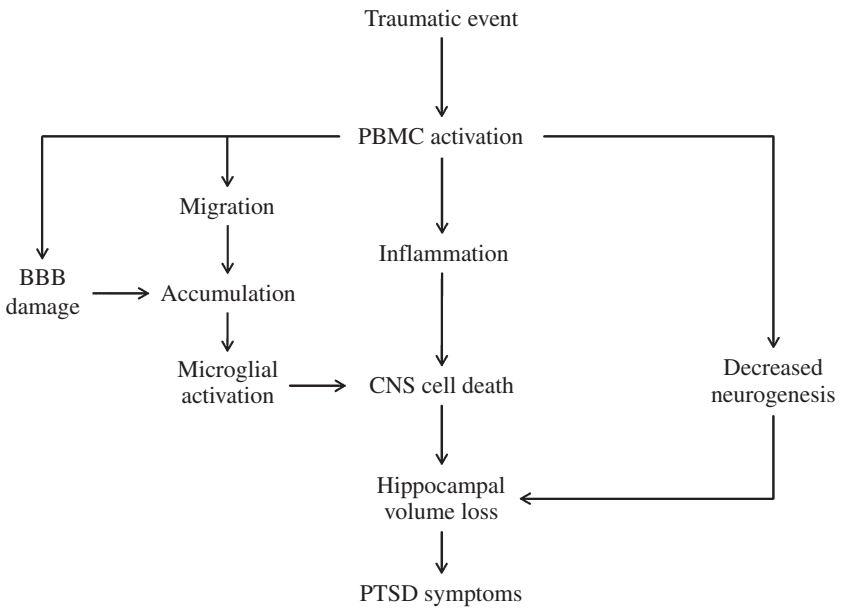

Fig. 1 The proposed mechanism of PBMCs affect on PTSD. PBMC, peripheral blood mononuclear cells; CNS, central nervous syndrome; BBB, blood–brain barrier; PTSD, post-traumatic stress disorder.

PBMCs increase with PTSD symptoms. In a study performed on trauma survivors, it was shown that there was a change in PBMC gene expression which correlated with the onset and severity of PTSD symptoms (Segman *et al.* 2005). Blood samples were taken upon arrival at the hospital as well as one and four months after the original traumatic event, and the gene expression of the PBMCs in the blood samples was determined utilizing microarrays. PTSD symptoms were evaluated using DSM IV diagnostic criteria at the same time as the blood samples were drawn. The change in PBMC gene expression correlated with the severity of PTSD symptoms. Moreover, genes associated with inflammation were of the most markedly altered in patients with PTSD symptoms. This study shows how the periphery can be involved in CNS disorders, and specifically demonstrates that PBMC activity correlates with PTSD. PTSD may involve a complex interplay between the CNS and the activity of specific circulating PBMC populations, but more studies need to be done to clarify this concept.

A study performed with soldiers before a parachute jump provides an example of how chronic stress can affect PBMC function in humans. In this study, Aloe *et al*. (1994) analyzed the blood of individuals the night before parachuting and 20 min after landing and compared the levels with those of controls. Although the stress of jumping from an airplane may appear acute, soldiers in paratrooper school are likely undergoing chronic stress because of the continuous training prior to executing the actual parachute jump. In plasma, it was found that nerve growth factor (NGF), cortisol, and adrenocorticotropic hormone were increased before and after landing. NGF receptor and tyrosine kinase receptor type 1 were also elevated in the PBMCs of the soldiers who jumped, indicating that PBMCs were activated. There appears to be a temporal pattern of activation in this study where some markers were elevated the night prior to the jump and other were elevated upon landing. These data suggest that NGF may be part of a PBMC mobilization/preparatory response to an approaching stressor. Activated PBMCs, possibly like those described here, may aid in the defense against possible infection encountered during fight or flight. Re-experiencing stressful events is a key symptom of PTSD, and it may be an important source of frequent and chronic stress. Chronically elevated serum levels of activated PBMCs may be a key link in the mechanism of PTSD.

The impact of activated PBMCs on human CNS homeostasis is not known, but most experiments performed in animal models suggest that PBMCs may exacerbate existing CNS injuries through inflammation. The spleen, an important source of PBMCs, appears to be involved in hyperinflammatory responses after neurological insults. In a recent study, it was shown that rats without spleens had reduced numbers of activated macrophages, neutrophils and microglia in comparison with those with spleens (Ajmo *et al.* 2008). After an ischemic stroke was induced, the rats with spleens showed more CNS tissue damage caused by inflammation compared with those without spleens. In other words, the presence of more PBMCs may exacerbate CNS damage through a proinflammatory response. This study may also have implications for other CNS disorders.

Lymphocytes, a type of PBMC, are involved in many different aspects of inflammation, infection, and disease. Similar to Ajmo *et al.* (2008), another study demonstrated

that the number of lymphocytes correlates with CNS damage. After inducing ischemia in rats, it was found that after 24 h, severely immunodeficient mice, which lack T lymphocytes, had a smaller infarct region than animals with intact immune systems (Hurn *et al.* 2007). This role of T lymphocytes may not be limited to infarction.

Recent data suggest that T lymphocytes play an important role in the CNS response to stressors, and this may present a mechanism for CNS damage after experiencing stress that leads to PTSD. A study by Lewitus *et al.* (2008) found that T lymphocytes infiltrate the blood brain barrier (BBB) after bouts of stress. The infiltration was associated with an increase of intercellular adhesion molecules (ICAM)-1 on the surface of the BBB. ICAM-1 is known to increase migration of cells into the CNS through the BBB. This study suggested that choroid plexi adjacent to hippocampal regions demonstrate the highest increase in ICAM-1 surface expression and adherence/infiltration of T lymphocytes. This may allow accumulation of T lymphocytes within the CNS after a single stress exposure, and more T lymphocytes can accumulate after severe or longer duration stress exposures. It is not known whether or not this type of cellular accumulation has an effect on PTSD symptoms. The ligand for ICAM-1, lymphocyte function-associated protein is displayed on the surface of lymphocytes, macrophages, and neutrophils, where it binds and adheres to ICAM-1. It was shown that blocking this interaction can reduce stress-induced inflammation (Joachim *et al.* 2008), so it is plausible that this receptor/ligand combination can be augmented to inhibit further CNS degradation.

One study found that lymphocytes isolated from PTSD patients exhibited decreased intracellular glucocorticoid receptors levels compared with controls (Gotovac *et al.* 2003), which indicates that lymphocytes may affect PTSD. Moreover, it has been shown that PTSD symptoms correlate with T lymphocyte counts and activation. In a similar study, war veterans diagnosed with PTSD were compared with controls with no war experience, and the number of circulating T lymphocytes were shown to be higher in subjects with PTSD (Vidovic *et al.* 2007).

In contrast to the evidence of negative effects of lymphocytes, a study showed that patients with a history of PTSD have decreased numbers of T lymphocytes (Kawamura *et al.* 2001). In this study, subjects diagnosed with PTSD according to the DSM IV were compared with controls that had a similar history of stress but without the signs and symptoms of PTSD. The results showed that interferon-γ and interleukin (IL)-4 are indicators of T helper cell type 1 and type 2 activities, respectively, were lower in patients with PTSD when compared with controls. This contrasts with the Vidovic *et al.* (2007) study, which showed that patients with PTSD had more T lymphocytes, demonstrating how little is known about T lymphocyte activity in PTSD patients. These discrepancies may be associated with the time of blood extraction; cell counts affected by stressors may have temporal patterns, such as those seen during mechanical trauma or infections. For example, lymphocyte counts may be higher immediately after traumatic incidents and may decrease significantly, even below baseline, afterwards, but studies to analyze these factors remain elusive.

The abovementioned study by Lewitus *et al.* also provides evidence of a possible T lymphocyte function for recovery from stress. In this study, mice were immunized against a 20 amino acid section of the myelin oligodendrocyte glycoprotein (Lewitus *et al.* 2008). Myelin oligodendrocyte glycoprotein is located on the surface of some oligodendrocytes and the outer surface of myelin. The authors found that the T lymphocyte migration to the CNS after immunization was associated with an increase in brain-derived neurotropic factor in the dentate gyrus of the hippocampus. The immunized animals exhibited reduced anxiety and were found to be more resilient to stress. Cellular accumulation in the CNS may be beneficial for adult neurogenesis, memory formation, and anxiolysis, but it is unknown which cell populations can deliver these favorable effects compared with the unfavorable effects. T lymphocyte activity is likely involved in the stress response, resiliency, and possibly PTSD, but more research needs to be conducted to find out how these cells and other PBMCs affect the course of PTSD. To our knowledge PTSD is solely a human disorder, but animal studies, such as the abovementioned, provide important evidence to support a mechanistic link between PBMCs and PTSD.

T lymphocytes may have multiple roles in the development of PTSD, and some specific actions may rely upon the particular T lymphocyte population that is activated. For example, T regulatory cells ($CD4^+$ $CD25^+$ cells) have been shown to aid in the reduction of anxiety symptoms in an animal model (Cohen *et al.* 2006). The T regulatory cells were collected from mice that were immunized against myelin basic protein. The actions of these T lymphocytes unlike others may benefit PTSD, but more research is needed to differentiate different T lymphocyte effects.

In further support of the beneficial actions of PBMCs, the mononuclear fraction of human umbilical cord blood cells and the mononuclear fraction of rat bone marrow progenitors appear to gain some of the characteristics of neurons when transplanted into rat brains (Walczak *et al.* 2004). After these cells were injected into brain parenchyma, a few cells were found to express nestin and endocortin, both markers of neural progenitor cells. Adult neurogenesis associated with effective treatments and symptoms may help restore CNS connectivity after a bout with PTSD, traumatic brain injury (TBI), or other neurodegenerative disease. In adults, neuroblasts that arise from the subventricular zone of the hippocampus were shown to migrate along a specific pathway, via the rostral migratory stream adhering via β1-integrin connection to rostral migratory stream laminin along

the surface of glia (Belvindrah *et al.* 2007). Walczak and colleagues also showed that some injected mononuclear cells migrated along this tract toward the olfactory bulb, a behavior similar to newly arisen neuroblasts (Walczak *et al.* 2004). This suggests that PBMCs may play a direct role in adult neurogenesis, a role that is beneficial to CNS homeostasis. Therefore, it is important to sustain or increase neurogenic functions when attempting to inhibit the PBMC functions that appear to be damaging and neurodegenerative.

Inflammation is defined as a collection of symptoms such as edema, loss of function, pain, elevated temperature, and redness. Inflammation is highly complex, so the aspects of inflammation most specific to neurodegeneration will be the focus of this review. Inflammation contributes to CNS cell death and is linked to the development of many CNS diseases and disorders including; Alzheimer's disease (Maccioni *et al.* 2009), Parkinson's disease (Hirsch and Hunot 2009), spinal cord injury (Chan 2008), multiple sclerosis (Frohman *et al.* 2008), and it may also contribute to PTSD. Importantly, this is a mechanism by which the periphery can affect CNS function.

Inflammatory processes have been correlated with emotion, and some data show that the relationships exist between personality types and the cytokine levels which control the level of inflammation. For example, in a study by Mommersteeg *et al.* (2008) individuals who are hostile appear to have more inducible inflammatory activity. The levels of 11 cytokines and chemokines were determined and compared with the hostility score of each subject. The levels of inflammatory markers correlated with the hostility score. This subsequent increase in inflammation may translate into diseases such as cardiovascular disease and a similar mechanism may contribute to CNS damage reported in PTSD. Work performed in rats has shown that stress may cause increased CNS damage after an inflammatory stimulus (de Pablos *et al.* 2006). In this study, inflammation was induced in stressed and non-stressed rats and inflammatory markers were subsequently measured. It was shown that stress strengthens the changes by the induced inflammation including an increase in proinflammatory cytokines and a decrease in the number of neurons. This study and others show that a growing body of descriptive data suggests that stress, anxiety, and inflammation are correlated, but empirical studies demonstrating cause-and-effect relationships are lacking.

PBMCs escape the circulating blood by way of attaching to adhesion molecules, rolling, and migration (van Buul and Hordijk 2004). The migration through the BBB endothelium and subsequent tissue layers toward chemoattractive substances such as monocyte chemotactic protein-1 and stromal cell-derived factor-1 (SDF-1) may cause additional injury. SDF-1 (or CXCL12), is highly expressed in damaged or hypoxic tissues (Ceradini *et al.* 2004). SDF-1 is a powerful chemoattractive substance for PBMCs via its receptor CXCR4. These functions allow PBMCs to enter inflamed and injured regions, such as may occur in the CNS after injury from TBI, spinal cord injury, stroke, or infection.

Although chemoattractive substances increase the number of PBMCs that migrate through the BBB, more PBMCs can transverse a BBB with issues. A malfunctioning BBB can allow for the accumulation of PBMCs in the brain parenchyma under normal conditions, which damage CNS tissue during their migration (Wunder *et al.* 2009). It has also been suggested that plasma proteins can induce BBB damage (Stolp and Dziegielewska 2009), and additional damage from migrating cells and their secreted products can lead to a further decline in CNS tissue health. An example of substances that can induce such damage are the matrix metalloproteinases, a class of enzymes (Rosenberg 2009). These proteins degrade intercellular linkages that are important for BBB integrity, and it is this an action by which the BBB and surrounding CNS tissue can be damaged.

Another mechanism which CNS cell death occurs is by microglia activity, which affects the course of tissue loss and patient function in several neurodegenerative diseases (Liu and Hong 2003). Microglia can damage surrounding tissue via several mechanisms which include release of cytokines, chemokines, other toxic substances, and migration (Raivich *et al.* 1999; Stoll *et al.* 2000); this role is shared with monocytes and macrophages in the progression of other inflammatory diseases in the periphery.

The CNS is populated with microglia during embryogenesis, and relatively little repopulation of CNS microglia occurs during adulthood (Chan *et al.* 2007). Some recent findings demonstrate that adult CNS microglial repopulation may have a significant effect on CNS function (Bechmann *et al.* 2005), suggesting that these few cells have large consequences for PTSD. In this study, the authors showed that PBMCs can infiltrate inflamed CNS tissue and acquire the microglial phenotype. The entorhinal cortex projects axons into the hippocampus; when severed in rodents, these axons degrade. Using this animal model, Bechmann and colleagues found that PBMCs migrated through the BBB and into the area of axonal degradation and injury. The authors concluded that the damage and axonal degradation were sufficient to induce release of pro-inflammatory substances. Unfortunately, no mechanistic investigation of PBMC recruitment or function followed the proposal of this hypothesis. However, with the growing body of literature, we can deduce that PBMCs may be involved in the neuronal damage that occurs during PTSD and other neurodegenerative diseases.

CD14 is a marker of microglia and when present in the CNS, is associated with decreased CNS function after TBI and ischemic damage (Beschorner *et al.* 2002a; b). This is evidence of microglial involvement in recovery from neurological insults. Although microglia can damage CNS tissue, they also protect against encephalitis and invasion of foreign

substances past the BBB. Modulation of their activity may be the key to reducing CNS inflammatory activity and altering the course of neuronal cell death. More research should be done to investigate microglial activity after stress and/or PTSD. Evidence of successful pharmaceutical inhibition of PBMC/microglia function was reported in an animal model of blast-induced TBI (Moochhala *et al.* 2004). In this investigation, the activity of inducible nitric oxide synthase (iNOS) was inhibited by administration of aminoguanidine. The rats with peripherally administered aminoguanidine did not have as many blast-related injuries or functional impairments. Interestingly, it appeared to not matter if the aminoguanidine was administered before or after the injury. As iNOS is only known to be expressed in immune cells such as PBMCs (Chabrier *et al.* 1999), these findings reinforce the role of PBMCs in neurodegeneration. PBMC involvement in CNS tissue loss may be activated after bouts of stress, and evidence suggests that we may be able to alter PBMC function to reduce neurodegeneration.

Although PTSD may be caused by an increased level of neuronal death, it may also be caused by a decreased level of neuronal cell growth. Selective serotonin reuptake inhibitors, which are used as a treatment for PTSD symptoms, have been shown to increase neurogenesis (Santarelli *et al.* 2003). Therefore, neurogenesis may counteract the neuronal cell death in PTSD patients. Studies performed in animal models suggest that PTSD/traumatic events decrease neurogenesis. Recently, it was shown that rats exhibit PTSD-like behavior after treatment with inescapable tail shock, which was dispensed as a chronic stressor (Kikuchi *et al.* 2008). In addition to PTSD-like behaviors, stressed rats had less bromodeoxyuridine (a marker for replicating DNA) incorporated into their hippocampal subgranular zone, further indicating that chronic stress correlates with decreased neurogenesis. Neurogenesis within the hippocampus occurs from the subgranular zone of the dentate gyrus. Currently, there is a lack of postmortem neuropathological studies on human PTSD populations to show more specific morphological and histological details of hippocampal atrophy or amygdaloid hypertrophy; thus, this is an area of great interest and demand.

The loss of CNS tissue during the development of PTSD symptoms may be directed by inflammation, microglial activation, and decreased neurogenesis. Another possible reason for CNS volume loss in PTSD patients is apoptosis. Cytokines including tumor necrosis factor-alpha (TNF-α), IL-1, and interferon-γ can act on their receptors to initiate apoptosis and subsequent neurodegeneration (Hitomi *et al.* 2008). This is one more possible link between the periphery and PTSD.

PTSD patients have reduced hippocampal volume when compared with healthy patients. This has also been documented in individuals with a traumatic childhood experience (Stein *et al.* 1997). A meta-analysis reported that the hippocampi in PTSD patients are nearly 7% smaller than controls (Smith 2005), while some individual studies have reported over twice that level of reduction (Gurvits *et al.* 1996; Hedges *et al.* 2003). A few reports may suggest that a small hippocampus pre-disposes people to PTSD, but at this time, the majority of the literature favors hippocampal atrophy as a consequence of PTSD or chronic stress (Sapolsky 2000; Bremner *et al.* 2008). The hippocampal tissue loss associated with PTSD may be due to several physiological mechanisms, microglial activation, inflammation and decreased neurogenesis. Therefore, a balance of tissue growth and atrophy may be part of the homeostasis of the hippocampus. In PTSD and under chronic stress conditions, this homeostatic ratio may become unbalanced, resulting in loss of tissue volume.

Potential biomarkers for PTSD

Biomarkers for PTSD (Table 1) are an area of continuous interest because they may provide some insight into the mechanism and an objective diagnosis. Objective diagnosis of PTSD is important because it may provide improved detection for a disease that is highly stigmatized and misdiagnosed among service members (Gaylord 2006). The markers associated with other CNS disorders can give some insight into which will be most effective as diagnostic tools. Eight potential PTSD biomarkers are reviewed below and many more will follow in the future. There may not be a single biomarker, but rather a combination that will provide a conclusive diagnosis of PTSD.

Neuropeptide-Y

Neuropeptide-Y (NPY), a sympathetic co-transmitter and hormone, is present in the circulation, and it is present at higher concentrations after sympathetic activation, supporting the connection between the sympatho-adrenomedullary system, bone marrow cells, and circulation (Zukowska-Grojec 1995). Moreover, NPY is important for the proper functioning of PBMCs. NPY is detectable in PBMCs, and it is altered during inflammation (Holler *et al.* 2008). NPY has a bimodal effect on T lymphocyte function. It mitigates the activity of mature T cells and, conversely, NPY is integral to mature T lymphocyte development (Wheway *et al.* 2005). NPY activities are carried out through multiple receptors, and NPY receptors modulate release of catecholamines via Y1 receptors (Cavadas *et al.* 2006) and pre-synaptic Y2 autoreceptors (Potter and Tripovic 2006). The actions of NPY and its multiple receptors are gaining importance in the field of PTSD, where NPY might act as an anxiolytic. Circulating NPY levels have been associated with more positive outcomes to stress in some studies, further supporting NPY's function as an anxiolytic (Morgan *et al.* 2002). Circulating levels of NPY may be indicative of CNS levels and sympatho-adrenomedullary system tone. NPY has multiple

Table 1 Biomarkers that may indicate development of PTSD symptoms

Biomarker	Human gene	Findings	Known functions	References
NPY	*NPY*	Released from sympathetic neurons during chronic and sustained stress	Is mitogenic and has pleiotropic effects on peripheral cell function	Zukowska-Grojec (1995), Wheway *et al.* (2005)
CD14	*CD 14*	In the CNS, associates with TBI, stroke, and microglial activity	An extracellular surface receptor that initiates an innate immune response	Beschorner *et al.* (2002a,b)
MRP8; S100A8	*S100A8*	TBI patients have elevated number in CNS tissue	Marker of microglia and macrophages. As a number subunit of calprotectin, it functions to stem bacterial growth by binding divalent cations	Beschorner *et al.* (2000)
pll; S100A10	*CLP 11*	Lower levels were found in PBMCs from PTSD patients compared with controls and other mental disorders	Functions as an adapter protein, and it traffics the serotonin receptor 5HT IB to the plasma membrane	Svenningsson *et al.* (2006), Su *et al.* (2009)
TNF-a	*TNF*	Associated with PTSD	Wide range of effects, including apoptosis. A very potent initiator of the inflammatory response	Lorz and Mehmet (2009)
IL-1	*ILIA* and *IL1B*	Elevated in PTSD patients	Released from injured or inflamed tissue, and causes a wide range of effects, including local and systemic inflammation and apoptosis	Spivak *et al.* (1997), Tucker *et al.* (2004)
IL-6	*IL-6*	Elevated in PTSD patients	Pleiotropic; initiates acute phase response and decreases some aspects of inflammation	Maes *et al.* (1999), von Kanel *et al.* (2010)
Gsα	*GNAS1*	Higher levels in lipid rafts associate with depression and suicide	This protein functions in signal transduction, communicating activity from receptors to intracellular molecules	Donati *et al.* (2008)

This table summarizes the biomarkers that may indicate development of PTSD. Corresponding references also indicated. PTSD, post-traumatic stress disorder; TBI, traumatic brain injury; IL, interleukin; MRP, myeloid-related protein; NPY, neuropeptide-Y; PBMC, peripheral blood mononuclear cells; TNF; tumor necrosis factor.

functions, and it associates with many diseases. Moreover, NPY appears to contribute to resiliency, and its roles in peripheral cell activity, inflammation, and chronic stress suggest that we further examine the role of NPY in PTSD.

CD14

Extensive work by Beschorner and colleagues showed that biomarkers may associate with PBMC/microglia-induced inflammation and damage after CNS insults. In a study of 18 stroke victims, follow-up neuropathological analysis showed that the number of $CD14^+$ (read as 'CD14-positive') cells had increased in local tissue (Beschorner *et al.* 2002b). Co-staining experimentation showed that microglia ($CD68^+$) displayed surface CD14, but no CD14 was found on T lymphocytes ($CD3^+$), astrocytes ($GFAP^+$), or endothelial cells ($CD34^+$). In conjunction with Toll-like receptor 4, CD14 is a co-receptor for bacterial membrane components. Activation of CD14 stimulates an innate immune response. It is activated in the CNS during bacterial meningitis, but it also functions as a good marker of microglial activity in the CNS.

In a similar investigation, TBI patients showed that $CD14^+$ cell counts within the CNS increased over the week following the TBI-inducing incident and remained elevated for weeks afterward. Neuropathological evaluation of 25 cases and five controls showed that $CD14^+$ cell counts are elevated in the region of the lesion and the surrounding area. $CD14^+$ cells appeared to accumulate within 1–2 days of the injury (Beschorner *et al.* 2002a). These findings were replicated in another study that focused on ischemic stroke and showed again that $CD14^+$ cells were elevated in and around the infarct lesion area (Beschorner *et al.* 2002b). The authors recommended that anti-CD14 therapies be considered for CNS inflammatory diseases. Studies by other groups have supported this hypothesis, because it was shown that

soluble CD14 in cerebral spinal fluid is elevated after CNS inflammatory states such as multiple sclerosis (Kraus *et al.* 2000), human immunodeficiency virus dementia (Fischer-Smith *et al.* 2001), and meningitis (Cauwels *et al.* 1999).

Myeloid-related protein-8

Another protein, myeloid-related protein-8 (MRP8) (S100A8), previously has been implicated as a potential biomarker for TBI (Beschorner *et al.* 2000). Beschorner and colleagues' work showed that MRP8 microglia accumulate after TBI in injured CNS tissue. MRP8 is part of an immune response; it binds free divalent cations and inhibits the growth of bacteria (Corbin *et al.* 2008). The studies on CD14 and MRP8 suggest that microglia, macrophages, and monocytes play an important role in TBI and stroke, and that some biomarkers are specific to CNS inflammation. Although these markers correlate well with stroke and TBI, it is unknown if they correlate with PTSD symptoms. Inflammation may exacerbate PTSD-related neurodegeneration, and more studies should be conducted on these biomarkers to investigate mechanistic links between microglial activity, inflammation, and PTSD.

p11

p11 (S100A10) messenger RNA is a potential biomarker for PTSD. In a recent investigation by Su *et al.* (2009), PBMC p11 levels were examined in PTSD, major depressive disorder, bipolar disorder, and schizophrenia patients and compared with controls (2009a). Real-time PCR, cortisol levels in blood and saliva, using radioimmunoassay were compared. PTSD patients had lower p11 messenger RNA in PBMCs than controls. The other disorders that were examined had significantly higher p11 levels than controls, suggesting that PTSD may be particularly associated with specific alterations in p11 expression levels. Interestingly, basal levels of cortisol of PTSD patients were not statistically different from controls, thus suggesting that p11 gene expression in PBMCs may function as a specific marker for PTSD.

p11 has been shown to affect depressive states. In a rodent model, it increased after antidepression treatments and was found to cause increased numbers of the serotonin receptor 5-hydroxytryptamine receptor 1B (Svenningsson *et al.* 2006). In this study, p11 functions as the adaptor protein for 5-hydroxytryptamine receptor 1B. Behaviorally, it was shown that p11-null mice exhibit the symptoms of depression. To our knowledge, p11 does not appear to have a role in inflammation. It appears to form complexes with annexin A2, and they function together to traffic proteins to membranes. More relevant, its presence in PBMCs presents a potential pathway for treatment of CNS disorders with peripheral cells. As discussed earlier, PBMCs can diffuse to regions of injury, and increased concentration of p11 may, in turn, increase seratonergic signaling. Therefore, p11 and other biomarkers should have the potential to provide diagnostic information and possible mechanistic insight, but more work is needed.

Tumor necrosis factor-alpha

TNF-α has an important role in immunity and it can eliminate tumors when present at high enough concentrations (Pennica *et al.* 1984; Shirai *et al.* 1985). In addition to these beneficial effects, there are many known pathophysiological effects of TNF-α in the CNS. The release of TNF-α from activated macrophages/microglia and subsequent cell death have been suggested to contribute to the mechanisms of Alzheimer's Disease, Parkinson's Disease, Multiple Sclerosis, and ischemic stroke lesion formation (Lorz and Mehmet 2009). TNF-α activates cell surface receptors on neurons and glia of the CNS, which can initiate the apoptotic process (Idriss and Naismith 2000). TNF-α also causes injury through increased activation of iNOS synthase and subsequent release of damaging amounts of nitric oxide (Gonzalez-Gay *et al.* 2009).

Interleukins

IL-1 may also be an important link between cellular action and the hippocampal volume loss associated with PTSD. In a study, serum IL-1β was elevated in (n = 19) PTSD patients and (n = 19) controls, and IL-1β levels also correlated with length of PTSD symptoms (Spivak *et al.* 1997). Another study showed similar findings. Patients (n = 58) had higher IL-1β levels than controls (n = 21) and were treated with SSRIs. After treatment, levels were lower than initial serum concentrations (Tucker *et al.* 2004). Thus, it appears that IL-1 may be an important marker for PTSD, but more studies should focus on possible mechanistic links between hippocampal cell death and PTSD.

IL-1 and TNF-α are released from PBMCs and other immune cells. Peripheral cytokine actions during inflammation are very complex. The actions of other cytokines such as IL-10 and IL-4 appear to be anti-inflammatory and thus possibly anti-neurodegenerative. In contrast, the actions of IL-6 are pleiotropic, presenting both anti- and pro-inflammatory properties (Tilg *et al.* 1997). In one study, PTSD patients with depression had higher levels of IL-6 than control patients, and patients with PTSD and no depression (Maes *et al.* 1999). A more recent study showed that PTSD patients have higher levels of IL-6 than control patients (von Kanel *et al.* 2010). These studies support the association between cytokines and PTSD.

Gsα

This protein subunit is vital for G-coupled protein receptor function. It was hypothesized that Gsα plays a role in PTSD symptomology (Gurguis *et al.* 1999), but only recently has its role become more understood. Recent work has shown the close linkage between Gsα and suicide and depression (Donati *et al.* 2008). Gsα migrates from the lipid rafts to the

membrane regions with more access to adenylate cyclase, and depressed individuals appear to have more Gsα in their lipid rafts than non-depressed. This molecule's presence in lipid rafts can be used as an indicator for G-coupled protein receptor functions, which appear to play important roles in PTSD symptoms. Therefore, studies need to be performed assess the effectiveness of this potential diagnostic tool for PTSD.

Glucocorticoids

A large body of evidence suggests that elevated glucocorticoids contribute to hippocampal volume reduction (Mc Ewen 1997, 2001). In particular, chronically elevated glucocorticoids have been shown to increase susceptibility to excitatory death (Roy and Sapolsky 2003). Much work has been performed from this perspective, and most of this has been effectively reviewed elsewhere (Sapolsky *et al.* 2000). The use of glucocorticoids as a PTSD biomarker should be explored further.

Conclusion

As long as a diagnosis for PTSD is elusive, treatment remains a serious problem (McAllister 2009). An important issue will be to define which populations of PBMCs are associated with beneficial and negative effects in PTSD patients. Dichotomous actions of PBMCs may reveal potential pathways of PTSD treatment with specific cell populations.

TBI is categorized as either severe or mild based on the initial symptoms following the trauma (Park *et al.* 2008). Mild TBIs are difficult to diagnose because they can be caused by minor acceleration-deceleration forces. Symptoms of TBI can vary, but some common symptoms are headache, tinnitus, dizziness, lack of concentration, and memory problems.

Diagnosing PTSD is a difficult task, but it is more daunting to effectively diagnose TBI versus PTSD (Hoge *et al.* 2008; Elder and Cristian 2009). Therefore, many individuals can be misdiagnosed, indicating that there is a current need for specific biomarkers to differentiate these two syndromes. In military populations, there is a recent increase in the rate of blast TBIs. An important indicator for blast TBI appears to be a perforated eardrum (Xydakis *et al.* 2007). Auditory and vestibular function, including tinnitus, can also be an important marker for TBI (Lew *et al.* 2007; Fausti *et al.* 2009). The finding that tinnitus is present among PTSD populations (Fagelson 2007) implies that more research should focus on auditory and vestibular markers to differentiate between TBI and PTSD. Also, diffuse axonal injury, areas of white matter damage, can be present in some TBI cases and be detected with the use of MRI via diffusion tensor imaging (Ducreux *et al.* 2005). Diffuse axonal injury appears to be a delayed process where axonal degeneration occurs in the days following axon severing, necrosis, or other insult. Some of the currently utilized assessments of TBI may also be indicators of PTSD. Therefore, biomarkers may be a clear and less expensive method to diagnose and differentiate these disorders.

In conclusion, stressful events may lead to PTSD, and diagnostic biomarkers are being investigated. Current findings suggest that PBMCs are involved in PTSD because concentrations of them and their secreted substances are associated with PTSD symptoms. Many PBMC-derived substances affect the function of CNS tissue and may contribute to hippocampal degradation during PTSD. The literature supports the potential to use externally derived cells for therapy; they can be modified, injected, home to the injured CNS tissue, and deliver payloads of neurotrophic factors or inhibitors of apoptosis. The overlap of PTSD and TBI symptoms and their comorbidity suggests that common mechanistic pathways may exist, and this makes it difficult to differentiate between them. At this time, more work should focus on PBMCs and their role in neurodegenerative diseases such as PTSD. Potential outcomes of this research may include better mitigation of neurodegeneration, PTSD, and sequelae of traumatic brain injury.

Acknowledgements

Report No. 10-27 supported by the Navy Bureau of Medicine and Surgery Psychological Health and Traumatic Brain Injury Program, under Work Unit No. 60818. The views expressed in this article are those of the author and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, or the U.S. Government. Approved for public release; distribution is unlimited. This research has been conducted in compliance with all applicable federal regulations governing the protection of human subjects. No human subjects were directly involved in this research. Special thanks to David Metzgar, PhD, for his assistance in preparation of this manuscript.

Conflict of interest

None declared.

References

Ajmo C. T., Vernon D. O. L., Collier L., Hall A. A., Garbuzova-Davis S., Willing A. and Pennypacker K. R. (2008) The spleen contributes to stroke-induced neurodegeneration. *J. Neurosci. Res.* **86**, 2227–2234.

Aloe L., Bracci-Laudiero L., Alleva E., Lambiase A., Micera A. and Tirassa P. (1994) Emotional stress induced by parachute jumping enhances blood nerve growth factor levels and the distribution of nerve growth factor receptors in lymphocytes. *Proc. Natl. Acad. Sci. USA* **91**, 10440–10444.

American Psychology Association (2000) Diagnostic and Statistical Manual of Mental Disorders. American Psychiatric Association, Washington, DC.

Bechmann I., Goldmann J., Kovac A. D., Kwidzinski E., Simburger E., Naftolin F., Dirnagl U., Nitsch R. and Priller J. (2005) Circulating monocytic cells infiltrate layers of anterograde axonal degeneration where they transform into microglia. *FASEB J.* **19**, 647–649.

Belvindrah R., Hankel S., Walker J., Patton B. L. and Muller U. (2007) Beta1 integrins control the formation of cell chains in the adult rostral migratory stream. *J. Neurosci.* **27**, 2704–2717.

Beschorner R., Engel S., Mittelbronn M., Adjodah D., Dietz K., Schluesener H. J. and Meyermann R. (2000) Differential regulation of the monocytic calcium-binding peptides macrophage-inhibiting factor related protein-8 (MRP8/S100A8) and allograft inflammatory factor-1 (AIF-1) following human traumatic brain injury. *Acta Neuropathol.* **100**, 627–634.

Beschorner R., Nguyen T. D., Gozalan F., Pedal I., Mattern R., Schluesener H. J., Meyermann R. and Schwab J. M. (2002a) CD14 expression by activated parenchymal microglia/macrophages and infiltrating monocytes following human traumatic brain injury. *Acta Neuropathol.* **103**, 541–549.

Beschorner R., Schluesener H. J., Gozalan F., Meyermann R. and Schwab J. M. (2002b) Infiltrating CD14 + monocytes and expression of CD14 by activated parenchymal microglia/macrophages contribute to the pool of CD14 + cells in ischemic brain lesions. *J. Neuroimmunol.* **126**, 107–115.

Bremner J. D., Elzinga B., Schmahl C. and Vermetten E. (2008) Structural and functional plasticity of the human brain in posttraumatic stress disorder. *Prog. Brain Res.* **167**, 171–186.

van Buul J. D. and Hordijk P. L. (2004) Signaling in leukocyte transendothelial migration. *Arterioscler. Thromb. Vasc. Biol.* **24**, 824–833.

Cauwels A., Frei K., Sansano S., Fearns C., Ulevitch R., Zimmerli W. and Landmann R. (1999) The origin and function of soluble CD14 in experimental bacterial meningitis. *J. Immunol.* **162**, 4762–4772.

Cavadas C., Cefai D., Rosmaninho-Salgado J. *et al.* (2006) Deletion of the neuropeptide Y (NPY) Y1 receptor gene reveals a regulatory role of NPY on catecholamine synthesis and secretion. *Proc. Natl. Acad. Sci. USA* **103**, 10497–10502.

Ceradini D. J., Kulkarni A. R., Callaghan M. J., Tepper O. M., Bastidas N., Kleinman M. E., Capla J. M., Galiano R. D., Levine J. P. and Gurtner G. C. (2004) Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat. Med.* **10**, 858–864.

Chabrier P. E., Demerle-Pallardy C. and Auguet M. (1999) Nitric oxide synthases: targets for therapeutic strategies in neurological diseases. *Cell. Mol. Life Sci.* **55**, 1029–1035.

Chan C. C. (2008) Inflammation: beneficial or detrimental after spinal cord injury? *Recent Pathol. CNS Drug Discov.* **3**, 189–199.

Chan W. Y., Kohsaka S. and Rezaie P. (2007) The origin and cell lineage of microglia: new concepts. *Brain Res. Rev.* **53**, 344–354.

Cohen H., Ziv Y., Cardon M., Kaplan Z., Matar M. A., Gidron Y., Schwartz M. and Kipnis J. (2006) Maladaptation to mental stress mitigated by the adaptive immune system via depletion of naturally occurring regulatory CD4 + CD25 + cells. *J. Neurobiol.* **66**, 552–563.

Corbin B. D., Seeley E. H., Raab A. *et al.* (2008) Metal chelation and inhibition of bacterial growth in tissue abscesses. *Science* **319**, 962–965.

Donati R. J., Dwivedi Y., Roberts R. C., Conley R. R., Pandey G. N. and Rasenick M. M. (2008) Postmortem brain tissue of depressed suicides reveals increased Gs alpha localization in lipid raft domains where it is less likely to activate adenylyl cyclase. *J. Neurosci.* **28**, 3042–3050.

Ducreux D., Huynh I., Fillard P., Renoux J., Petit-Lacour M. C., Marsot-Dupuch K. and Lasjaunias P. (2005) Brain MR diffusion tensor imaging and fibre tracking to differentiate between two diffuse axonal injuries. *Neuroradiology* **47**, 604–608.

Elder G. A. and Cristian A. (2009) Blast-related mild traumatic brain injury: mechanisms of injury and impact on clinical care. *Mt Sinai J. Med.* **76**, 111–118.

Fagelson M. A. (2007) The association between tinnitus and posttraumatic stress disorder. *Am. J. Audiol.* **16**, 107–117.

Fausti S. A., Wilmington D. J., Gallun F. J., Myers P. J. and Henry J. A. (2009) Auditory and vestibular dysfunction associated with blast-related traumatic brain injury. *J. Rehabil. Res. Dev.* **46**, 797–810.

Fischer-Smith T., Croul S., Sverstiuk A. E. *et al.* (2001) CNS invasion by CD14 +/CD16 + peripheral blood-derived monocytes in HIV dementia: perivascular accumulation and reservoir of HIV infection. *J. Neurovirol.* **7**, 528–541.

Frohman E. M., Eagar T., Monson N., Stuve O. and Karandikar N. (2008) Immunologic mechanisms of multiple sclerosis. *Neuroimaging Clin. N. Am.* **18**, 577–588.

Gaylord K. M. (2006) The psychosocial effects of combat: the frequently unseen injury. *Crit. Care Nurs. Clin. North Am.* **18**, 349–357.

Gonzalez-Gay M. A., Garcia-Unzueta M. T., Berja A., Vazquez-Rodriguez T. R., Miranda-Filloy J. A., Gonzalez-Juanatey C., de Matias J. M., Martin J., Dessein P. H. and Llorca J. (2009) Short-term effect of anti-TNF-alpha therapy on nitric oxide production in patients with severe rheumatoid arthritis. *Clin. Exp. Rheumatol.* **27**, 452–458.

Gotovac K., Sabioncello A., Rabatic S., Berki T. and Dekaris D. (2003) Flow cytometric determination of glucocorticoid receptor (GCR) expression in lymphocyte subpopulations: lower quantity of GCR in patients with post-traumatic stress disorder (PTSD). *Clin. Exp. Immunol.* **131**, 335–339.

Gurguis G. N., Andrews R., Antai-Otong D., Vo S. P., Blakeley J. E., Orsulak P. J. and Rush A. J. (1999) Neutrophil beta2-adrenergic receptor coupling efficiency to Gs protein in subjects with post-traumatic stress disorder and normal controls. *Psychopharmacology (Berl)* **143**, 131–140.

Gurvits T. V., Shenton M. E., Hokama H. *et al.* (1996) Magnetic resonance imaging study of hippocampal volume in chronic, combat-related posttraumatic stress disorder. *Biol. Psychiatry* **40**, 1091–1099.

Hedges D. W., Allen S., Tate D. F., Thatcher G. W., Miller M. J., Rice S. A., Cleavinger H. B., Sood S. and Bigler E. D. (2003) Reduced hippocampal volume in alcohol and substance naive Vietnam combat veterans with posttraumatic stress disorder. *Cogn. Behav. Neurol.* **16**, 219–224.

Hirsch E. C. and Hunot S. (2009) Neuroinflammation in Parkinson's disease: a target for neuroprotection? *Lancet Neurol.* **8**, 382–397.

Hitomi J., Christofferson D. E., Ng A., Yao J., Degterev A., Xavier R. J. and Yuan J. (2008) Identification of a molecular signaling network that regulates a cellular necrotic cell death pathway. *Cell* **135**, 1311–1323.

Hoge C. W., McGurk D., Thomas J. L., Cox A. L., Engel C. C. and Castro C. A. (2008) Mild traumatic brain injury in U.S. Soldiers returning from Iraq. *N. Engl. J. Med.* **358**, 453–463.

Holler J., Zakrzewicz A., Kaufmann A., Wilhelm J., Fuchs-Moll G., Dietrich H., Padberg W., Kuncova J., Kummer W. and Grau V. (2008) Neuropeptide Y is expressed by rat mononuclear blood leukocytes and strongly down-regulated during inflammation. *J. Immunol.* **181**, 6906–6912.

Hurn P. D., Subramanian S., Parker S. M., Afentoulis M. E., Kaler L. J., Vandenbark A. A. and Offner H. (2007) T- and B-cell-deficient mice with experimental stroke have reduced lesion size and inflammation. *J. Cereb. Blood Flow Metab.* **27**, 1798–1805.

Idriss H. T. and Naismith J. H. (2000) TNF alpha and the TNF receptor superfamily: structure-function relationship(s). *Microsc. Res. Tech.* **50**, 184–195.

Joachim R. A., Handjiski B., Blois S. M., Hagen E., Paus R. and Arck P. C. (2008) Stress-induced neurogenic inflammation in murine skin skews dendritic cells towards maturation and migration: key role of intercellular adhesion molecule-1/leukocyte function-associated antigen interactions. *Am. J. Pathol.* **173**, 1379–1388.

von Kanel R., Begre S., Abbas C. C., Saner H., Gander M. L. and Schmid J. P. (2010) Inflammatory biomarkers in patients with posttraumatic stress disorder caused by myocardial infarction and the role of depressive symptoms. *Neuroimmunomodulation* **17**, 39–46.

Kawamura N., Kim Y. and Asukai N. (2001) Suppression of cellular immunity in men with a past history of posttraumatic stress disorder. *Am. J. Psychiatry* **158**, 484–486.

Kikuchi A., Shimizu K., Nibuya M., Hiramoto T., Kanda Y., Tanaka T., Watanabe Y., Takahashi Y. and Nomura S. (2008) Relationship between post-traumatic stress disorder-like behavior and reduction of hippocampal 5-bromo-2'-deoxyuridine-positive cells after inescapable shock in rats. *Psychiatry Clin. Neurosci.* **62**, 713–720.

Kraus J., Oschmann P., Engelhardt B., Bauer R., Schiel C., Kern A., Stolz E., Traupe A. and Dorndorf W. (2000) Soluble and cell surface ICAM-3 in blood and cerebrospinal fluid of patients with multiple sclerosis: influence of methylprednisolone treatment and relevance as markers for disease activity. *Acta Neurol. Scand.* **101**, 135–139.

Lew H. L., Jerger J. F., Guillory S. B. and Henry J. A. (2007) Auditory dysfunction in traumatic brain injury. *J. Rehabil. Res. Dev.* **44**, 921–928.

Lewitus G. M., Cohen H. and Schwartz M. (2008) Reducing post-traumatic anxiety by immunization. *Brain Behav. Immun.* **22**, 1108–1114.

Liu B. and Hong J. S. (2003) Role of microglia in inflammation-mediated neurodegenerative diseases: mechanisms and strategies for therapeutic intervention. *J. Pharmacol. Exp. Ther.* **304**, 1–7.

Lorz C. and Mehmet H. (2009) The role of death receptors in neural injury. *Front. Biosci.* **14**, 583–595.

Maccioni R. B., Rojo L. E., Fernandez J. A. and Kuljis R. O. (2009) The role of neuroimmunomodulation in Alzheimer's disease. *Ann. N Y Acad. Sci.* **1153**, 240–246.

Maes M., Lin A. H., Delmeire L., Van Gastel A., Kenis G., De Jongh R. and Bosmans E. (1999) Elevated serum interleukin-6 (IL-6) and IL-6 receptor concentrations in posttraumatic stress disorder following accidental man-made traumatic events. *Biol. Psychiatry* **45**, 833–839.

McAllister T. W. (2009) Psychopharmacological issues in the treatment of TBI and PTSD. *Clin. Neuropsychol.* **23**, 1338–1367.

McEwen B. S. (1997) Possible mechanisms for atrophy of the human hippocampus. *Mol. Psychiatry* **2**, 255–262.

McEwen B. S. (2001) Plasticity of the hippocampus: adaptation to chronic stress and allostatic load. *Ann. N Y Acad. Sci.* **933**, 265–277.

Mommersteeg P. M., Vermetten E., Kavelaars A., Geuze E. and Heijnen C. J. (2008) Hostility is related to clusters of T-cell cytokines and chemokines in healthy men. *Psychoneuroendocrinology* **33**, 1041–1050.

Moochhala S. M., Md S., Lu J., Teng C. H. and Greengrass C. (2004) Neuroprotective role of aminoguanidine in behavioral changes after blast injury. *J. Trauma* **56**, 393–403.

Morgan C. A. 3rd, Rasmusson A. M., Wang S., Hoyt G., Hauger R. L. and Hazlett G. (2002) Neuropeptide-Y, cortisol, and subjective distress in humans exposed to acute stress: replication and extension of previous report. *Biol. Psychiatry* **52**, 136–142.

de Pablos R. M., Villaran R. F., Arguelles S., Herrera A. J., Venero J. L., Ayala A., Cano J. and Machado A. (2006) Stress increases vulnerability to inflammation in the rat prefrontal cortex. *J. Neurosci.* **26**, 5709–5719.

Park E., Bell J. D. and Baker A. J. (2008) Traumatic Brain Injury: Can the consequences be stopped? *Canadian Med. Assoc. J.* **178**, 1163–1170.

Pennica D., Nedwin G. E., Hayflick J. S., Seeburg P. H., Derynck R., Palladino M. A., Kohr W. J., Aggarwal B. B. and Goeddel D. V. (1984) Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature* **312**, 724–729.

Potter E. K. and Tripovic D. (2006) Modulation of sympathetic neurotransmission by neuropeptide Y Y2 receptors in rats and guinea pigs. *Exp. Brain Res.* **173**, 346–352.

Raivich G., Jones L. L., Werner A., Bluthmann H., Doetschmann T. and Kreutzberg G. W. (1999) Molecular signals for glial activation: pro- and anti-inflammatory cytokines in the injured brain. *Acta Neurochir. Suppl.* **73**, 21–30.

Rosenberg G. A. (2009) Matrix metalloproteinases and their multiple roles in neurodegenerative diseases. *Lancet Neurol.* **8**, 205–216.

Roy M. and Sapolsky R. M. (2003) The exacerbation of hippocampal excitotoxicity by glucocorticoids is not mediated by apoptosis. *Neuroendocrinology* **77**, 24–31.

Santarelli L., Saxe M., Gross C. *et al.* (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* **301**, 805–809.

Sapolsky R. M. (2000) Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Arch. Gen. Psychiatry* **57**, 925–935.

Sapolsky R. M., Romero L. M. and Munck A. U. (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* **21**, 55–89.

Segman R. H., Shefi N., Goltser-Dubner T., Friedman N., Kaminski N. and Shalev A. Y. (2005) Peripheral blood mononuclear cell gene expression profiles identify emergent post-traumatic stress disorder among trauma survivors. *Mol. Psychiatry* **10**, 500–513.

Shirai T., Yamaguchi H., Ito H., Todd C. W. and Wallace R. B. (1985) Cloning and expression in Escherichia coli of the gene for human tumour necrosis factor. *Nature* **313**, 803–806.

Smith M. E. (2005) Bilateral hippocampal volume reduction in adults with post-traumatic stress disorder: a meta-analysis of structural MRI studies. *Hippocampus* **15**, 798–807.

Spivak B., Shohat B., Mester R., Avraham S., Gil-Ad I., Bleich A., Valevski A. and Weizman A. (1997) Elevated levels of serum interleukin-1 beta in combat-related posttraumatic stress disorder. *Biol. Psychiatry* **42**, 345–348.

Stein M. B., Koverola C., Hanna C., Torchia M. G. and McClarty B. (1997) Hippocampal volume in women victimized by childhood sexual abuse. *Psychol. Med.* **27**, 951–959.

Stoll G., Jander S. and Schroeter M. (2000) Cytokines in CNS disorders: neurotoxicity versus neuroprotection. *J. Neural Transm. Suppl.* **59**, 81–89.

Stolp H. B. and Dziegielewska K. M. (2009) Review: Role of developmental inflammation and blood-brain barrier dysfunction in neurodevelopmental and neurodegenerative diseases. *Neuropathol. Appl. Neurobiol.* **35**, 132–146.

Su T. P., Zhang L., Chung M. Y. *et al.* (2009) Levels of the potential biomarker p11 in peripheral blood cells distinguish patients with PTSD from those with other major psychiatric disorders. *J. Psychiatr. Res.* **43**, 1078–1085.

Svenningsson P., Chergui K., Rachleff I., Flajolet M., Zhang X., El Yacoubi. M., Vaugeois J. M., Nomikos G. G. and Greengard P. (2006) Alterations in 5-HT1B receptor function by p11 in depression-like states. *Science* **311**, 77–80.

Tilg H., Dinarello C. A. and Mier J. W. (1997) IL-6 and APPs: anti-inflammatory and immunosuppressive mediators. *Immunol. Today* **18**, 428–432.

Tucker P., Ruwe W. D., Masters B., Parker D. E., Hossain A., Trautman R. P. and Wyatt D. B. (2004) Neuroimmune and cortisol changes in selective serotonin reuptake inhibitor and placebo treatment of chronic posttraumatic stress disorder. *Biol. Psychiatry* **56**, 121–128.

Vidovic A., Vilibic M., Sabioncello A., Gotovac K., Rabatic S., Folnegovic-Smalc V. and Dekaris D. (2007) Circulating lymphocyte subsets, natural killer cell cytotoxicity, and components of hypothalamic-pituitary-adrenal axis in Croatian war veterans with posttraumatic stress disorder: cross-sectional study. *Croat. Med. J.* **48**, 198–206.

Walczak P., Chen N., Hudson J. E., Willing A. E., Garbuzova-Davis S. N., Song S., Sanberg P. R., Sanchez-Ramos J., Bickford P. C. and Zigova T. (2004) Do hematopoietic cells exposed to a neurogenic environment mimic properties of endogenous neural precursors? *J. Neurosci. Res.* **76**, 244–254.

Wheway J., Mackay C. R., Newton R. A., Sainsbury A., Boey D., Herzog H. and Mackay F. (2005) A fundamental bimodal role for neuropeptide Y1 receptor in the immune system. *J. Exp. Med.* **202**, 1527–1538.

Wunder A., Klohs J. and Dirnagl U. (2009) Non-invasive visualization of CNS inflammation with nuclear and optical imaging. *Neuroscience* **158**, 1161–1173.

Xydakis M. S., Bebarta V. S., Harrison C. D., Conner J. C., Grant G. A. and Robbins A. S. (2007) Tympanic-membrane perforation as a marker of concussive brain injury in Iraq. *N. Engl. J. Med.* **357**, 830–831.

Zukowska-Grojec Z. (1995) Neuropeptide Y. A novel sympathetic stress hormone and more. *Ann. N Y Acad. Sci.* **771**, 219–233.

REPORT DOCUMENTATION PAGE

1. REPORT DATE (DD MM YY)
22 02 10

2. REPORT TYPE
Journal submission

3. DATES COVERED (from – to)
Jul 2009-Jan 2010

4. TITLE
Cells, biomarkers, and posttraumatic stress disorder: Evidence for peripheral involvement in a central disease

5a. Contract Number:
5b. Grant Number:
5c. Program Element Number:
5d. Project Number:
5e. Task Number:
5f. Work Unit Number: 60818

6. AUTHORS
James A. Andrews, PhD, LT, MSC, USN

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Commanding Officer
Naval Health Research Center
140 Sylvester Rd
San Diego, CA 92106-3521

8. PERFORMING ORGANIZATION REPORT NUMBER
Report No. 10-27

9. SPONSORING/MONITORING AGENCY NAMES(S) AND ADDRESS(ES)
Commanding Officer
Naval Medical Research Center
503 Robert Grant Ave
Silver Spring, MD 20910-7500

Commander
Navy Medicine Support Command
P.O. Box 140
Jacksonville, FL 32212-0140

10. SPONSOR/MONITOR'S ACRONYM(S)
NMRC/NMSC

11. SPONSOR/MONITOR'S REPORT NUMBER(s)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Posttraumatic stress disorder (PTSD) is a serious disease that involves many different factors. Recently, studies have increasingly pointed toward the development of diagnostic assays for PTSD, and some of potential biomarkers are reviewed here. Additionally, the hypothesis that peripheral blood mononuclear cells (PBMCs) exacerbate PTSD is investigated and a mechanism is proposed. PBMCs include monocytes, macrophages, and lymphocytes, and their actions are complex, acting in concert with many factors to exert their effects. Several experimental animal models have described associations between neurological damage and PBMC activity. Data discussed from these models suggest that inflammatory activity may be increased in the central nervous system (CNS) during chronic stress, and some of these actions are mediated by PBMCs. Ironically, some aspects of PBMC function appear to protect against the symptoms of PTSD, so care should be taken when proposing to alter their activity. In conclusion, several biomarkers, including some cytokines and Gsα, appear to associate with disease states and PTSD. Neuropeptide-Y, a sympathetic co-transmitter and hormone, may exacerbate CNS tissue atrophy associated with PTSD, while providing beneficial anxiolytic effects. Further experimentation may offer therapeutic tools based on the connection between stress, PBMC function, and PTSD.

15. SUBJECT TERMS
PBMC, posttraumatic stress disorder, PTSD, inflammation

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	18a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Commanding Officer
UNCL	UNCL	UNCL	UNCL	11	18b. TELEPHONE NUMBER (INCLUDING AREA CODE) COMM/DSN: (619) 553-8429